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W81XWH-08-1-0508

TITLE:

"The Isolation and Characterization of Human Prostate Cancer Stem Cells"

PRINCIPAL INVESTIGATOR:

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CONTRACTING ORGANIZATION:

The University of Michigan, Ann Arbor, MI 48109

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

The overall objective of this proposal is to develop a durable cure for lethal prostate cancer through the elucidation of the role of cancer stem cells in the pathogenesis of the disease. During the past year, we have made the following significant findings/observations: i)3D culture of human prostate cancer cells with magnetic nanoparticles is not optimal for tumor initiation studies, ii) in vitro co-culture of human prostate cancer cells (established cell lines and primary patient samples) with human prostate fibroblasts hold promise as models of tumor initiation/cancer stem cell activity. We continue to optimize and validate our in vitro model of prostate cancer initiation to facilitate cancer stem cell discovery as well as drug targeting.

15. SUBJECT TERMS

Prostate cancer, stem cell, in vitro assay, cancer stem cell

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Introduction

The overarching goal of this proposal is to develop a durable cure for men with advanced prostate cancer through an improved understanding of the role of human prostate cancer stem cells in the pathogenesis of the disease. To this end, we have proposed the following specific aims: **1)** to identify and prospectively isolate prostate cancer stem cells from human prostate cancer tissue, **2)** to examine human prostate cancer cell lines, both primary and established, for cells that express cancer stem cell surface markers and the ability to determine therapy resistance *in vitro*, and **3)** to develop an *in vivo* model to assess human prostate cancer stem cell targeted therapy. The elucidation of the differential biology of cancer stem cells, versus the bulk population of cancer cells, has the potential to lead to the identification of novel therapeutic targets that aim to cripple the driving force behind lethal prostate cancer.

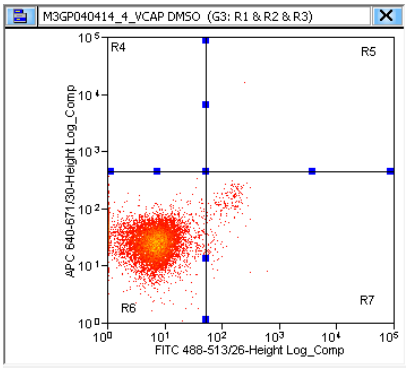
Body

From 29 JAN 2013 - 28 JAN 2014 our lab was involved in i) hiring personnel, ii) procuring necessary equipment and iii) obtaining all necessary institutional approvals to conduct laboratory research. This included submitting a University of Michigan human subjects IRB protocol as well as an animal research protocol. Once these were obtained they were submitted to DoD for approval. The process to obtain DoD approval for our animal research protocol has been somewhat lengthy and at the end of this period is awaiting DoD approval. An updated SOW was also submitted to the DoD and after several iterations was approved in September 2013.

Given that DoD approval of our animal protocol was just approved in March 2014, no significant progress was made on animal studies during this grant period.

The functional definition of a cancer stem cell is a cell that is treatment resistant. To this end, we treated androgen receptor (AR+) prostate cancer cells VCaP and C4-2 with the potent anti-androgen enzalutamide (MDV3100) in vitro and examined the surviving populations by FACS for expression of the putative cancer stem cell marker CD44. Notably, C4-2 cells, while AR+ are not AR dependent, and hence are considered castrate resistant prostate cancer (CRPC) like.

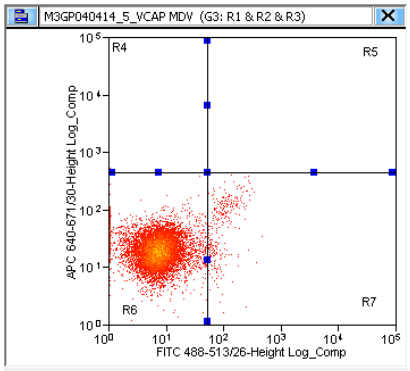
VCaP DMSO



Region	Count	% Hist	% All
Total	10000	100.00	24.96
R4	0	0.00	0.00
R5	1	0.01	0.00
R6	9791	97.91	24.43
R7	208	2.08	0.52

CD44+ population: 2.08%

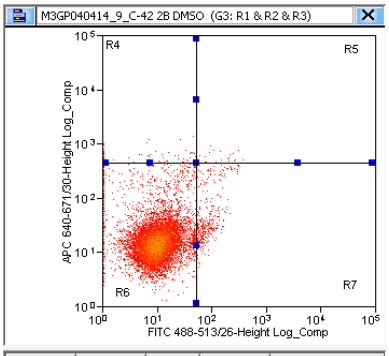
VCaP enza



Region	Count	% Hist	% All
Total	10000	100.00	19.52
R4	1	0.01	0.00
R5	0	0.00	0.00
R6	9702	97.02	18.93
R7	297	2.97	0.58

2.97%

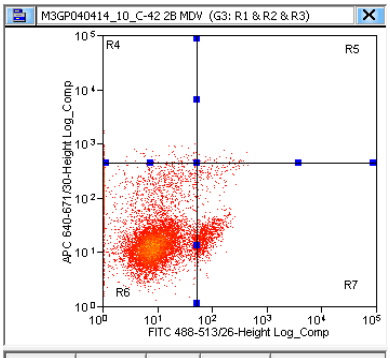
C4-2 DMSO



Region	Count	% Hist	% All
Total	10000	100.00	21.56
R4	55	0.55	0.12
R5	20	0.20	0.04
R6	9562	95.62	20.61
R7	363	3.63	0.78

CD44+ population: 3.36%

C4-2 enza



Region	Count	% Hist	% All
Total	10000	100.00	13.99
R4	80	0.80	0.11
R5	31	0.31	0.04
R6	8953	89.53	12.52
R7	936	9.36	1.31

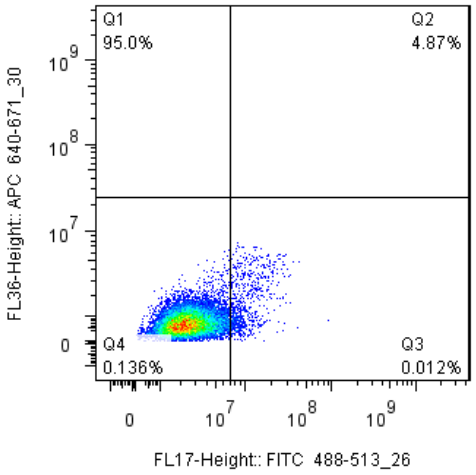
9.63%

These early studies suggest that enzalutamide resistant cells appear enriched for CD44+, particularly in CRPC like cells. We are testing this observation in multiple other lines and plan to do in vivo studies as well to see if systemic treatment (enza and others) results disease that is enriched in CD44+ cells.

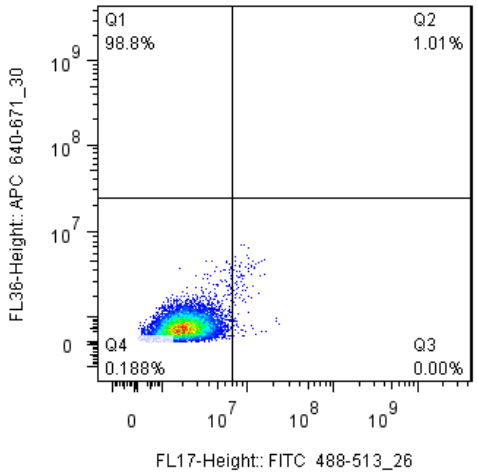
Given that we, and others, have had significant issues with taking fresh frozen tissues from radical prostatectomy specimens and generating tumors from single cell suspensions derived these samples, we are embarking on generating xenografts from chunks of tissue from radical prostatectomy specimens obtained from men with high grade disease (Gleason ≥8). After these have formed, we will treat animals with systemic therapies (e.g., enzalutamide) and examine refractory residual disease for stem cell markers. We will then validate our findings via correlation with treatment refractory samples in our vast tissue bank, inclusive of our warm autopsy cohort. We believe this approach will be more fruitful that our previous efforts.

Additionally, we have also begun testing the antibiotic compound salinomycin, a drug recently shown to target CD44+ cells (Gupta et al, Cell 2009, Ketola et al, British J Cancer, 2012).

VCaP DMSO



VCaP Salinomycin(10uM)



CD44+ population: 4.87%

1.06%

Scarce data exist on this compound in prostate cancer. We plan to test this compound in combination with other treatments (e.g., enza) in vitro and in vivo. Our hypothesis is that dual treatment in this way will stifle the emergence of a treatment resistant cell population.

Due to my changing of institutions twice during this award, please note that we have received an extension to complete this award thru February 2015.

Key Research and Training Accomplishments

Research accomplishments:
None this past year

Training accomplishments:

- Attend prostate cancer research seminars bi-weekly
- Attend monthly prostate SPORE research meetings monthly
- Attend UM Cancer Center research seminars monthly

Reportable Outcomes

1. Manuscripts
   None during this period relevant to this award

2. Funding

T32 in Urologic Oncology was submitted to NCI/NIH
1T32CA180984-01A1
“Advanced Training in Urologic Oncology”
Role: PI

Conclusion

From the work completed thus far, we conclude that i) 3D culture of human prostate cancer cells with magnetic nanoparticles is not optimal for tumor initiation studies, ii) in vitro co-culture of human prostate cancer cells (established cell lines and primary patient samples) with human prostate fibroblasts hold promise as models of tumor initiation/cancer stem cell activity.

References

None

Appendix

None